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Hepato-Splenic γδ T-Cell Lymphoma: A Rare Entity Mimicking the Hemophagocytic Syndrome

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Hepatosplenic γδ T-cell lymphoma is a rare histologic type of the peripheral T-cell lymphomas, clinically characterized by predominant involvement of liver and spleen, no or little adenopathy, and an often aggressive course; it affects mainly adolescents and young adults, with a male predominance. Postthymic T-cell malignancies are heterogeneous in their clinical and laboratory features. Among the γδ postthymic T-cell lymphomas, two distinct entities (cutaneous and hepatosplenic, respectively) are reported in the literature. The former shows predominant multiple involvement of the skin and subcutaneous tissue; it occurs mostly in older patients and the phenotype is CD3−, CD4−, CD8−. Because of the small number of reports, the course of the disease was unknown. The latter shows a clinical picture characterized by hepatosplenomegaly, no or little adenopathy, and sometimes systemic symptoms (fever, cytopenias likely due to hypersplenism); it presents a peculiar sinusoidal involvement of liver and spleen. The bone marrow histologic feature often reveals a little infiltration, especially sinusoidal and easily underestimated phenotype: CD2+, CD3+, CD7+, CD5−, CD4−, CD8−, CD44+. Few cases of this lymphoma associated by hemophagocytic syndrome are described (Sun, 1990; Kadin, 1981; Jaffe, 1983). We report a case of a young man with a rapid and fatal course in which the more important clinical feature was hemophagocytosis. The diagnosis of lymphoma was very difficult because of paucity of histologic involvement, and only the rearrangement of TCR γ chain gene by polymerase chain reaction on paraffin sections confirmed a clonal T-cell proliferation. Am. J. Hematol. 60:61–65, 1999. © 1999 Wiley-Liss, Inc.

Key words: hepato-splenic γδ T-Cell lymphoma; hemophagocytic syndrome; PCR

INTRODUCTION

Hepatosplenic γδ T-cell lymphoma is a rare histologic type of the peripheral T-cell lymphomas, clinically characterized by predominant involvement of liver and spleen, no or little adenopathy, and an often aggressive course; it affects mainly adolescents and young adults, with a male predominance [1]. Few cases of this lymphoma associated by hemophagocytic syndrome are described [2–4]. We report a case of a young man with a rapid and fatal course, in which the more important clinical feature was the hemophagocytosis. The diagnosis of lymphoma was very difficult because of paucity of histologic involvement, and was obtained by rearrangement of the T-cell receptor (TCR) γ-chain gene by polymerase chain reaction.

CASE REPORT

At the end of October 1995, a 23-year-old man, was admitted to the Department of Infectious Diseases with fever, anemia, hepatomegaly (4 cm), and splenomegaly (6 cm); no lymph nodes were palpable. Extensive search for bacterial, fungal, and viral sources of fever, including blood smears for malarial parasites, Widal-Weil-Felix test, hepatitis A, B, and C, cytomegalovirus (CMV), HIV, and brucella were all negative. Immunoglobulin

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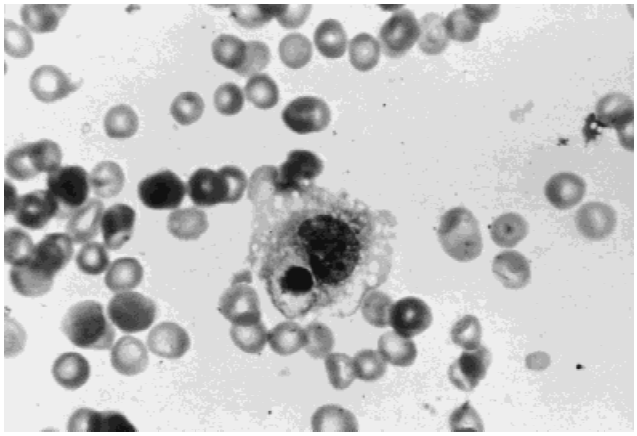

Fig. 1. Bone marrow aspirate: Cytoplasmatic presence of erythroblast in macrophage cell (magnification, ×1,000, oil immersion).

(Ig)G, but not IgM Epstein-Barr virus (EBV) antibodies were detected. Purified protein derivative of tuberculin was negative. Bone marrow cultures for leishmania were also negative. No antinuclear antibody and anti-DNA were found. The hematological parameters were: White blood cell, 5.7×10^9/L; platelets, 162×10^9/L; hematocrit, 21.4%; red blood cells, 2.39×10^{12}/L; Hb, 7.7 g/dL; reticulocytes, 15%; bilirubin, 1.7 mg/dL; lactate dehydrogenase, 206 UI/L; ferritin, 3,260 ng/mL, haptoglobin, 222 mg/dL, fibrinogen, 690 mg/dL; alkaline phosphatase, 98 IU/L; aspartate aminotransferase, 12 UI/L; and alanine aminotransferase, 14 UI/L. Chest X-ray was within normal limits. The patient was moved to the Division of Hematology; a new bone marrow aspirate and biopsy showed a very cellular specimen with myeloid hyperplasia, dyserythropoiesis, and dysmegakaryopoiesis; there was presence of hemophagocytic histiocytes (Fig. 1) but no infiltration of lymphoma.

Cytogenetic study, performed on medullary aspirate according to standard method, obtained an abnormal karyotype: 3/12 metaphases were 48, XY, del (3) (q21), +der (3) t (3; ?) q (21; ?) +6; bone marrow immunophenotype did not present clonality. Computed tomography scan of the abdomen and pelvis showed no lymphadenopathy and remarkable hepatosplenomegaly without focal lesions. Percutaneous liver biopsy was done; histologic findings showed no lymphoma infiltration. In December, after a new bone marrow biopsy and aspiration (chromosome analysis was abnormal in 10/30 metaphases), the patient underwent an exploratory laparotomy with splenectomy and multiple liver biopsies because of suspicion of lymphoproliferative disease; there was no involvement of lymph nodes. Specimens from spleen, liver, and bone marrow were fixed in formalin and processed according to standard technique; paraffin sections were stained with hematoxilin, eosin, and Giemsa for

TABLE I. Monoclonal Antibodies Tested*

Antibody	Major specificity	NHL cells reactivity
CD1	T cell, Langerhans cells	−
CD2	T cell	+
CD3	T cell	+
CD4	T helper, inducer	−
CD5	T cell	−
CD7	T cell	+
CD8	T suppressor, cytotoxic	−
CD10	B cell	−
CD15	Monocyte	−
CD19	B cell	−
CD21	B cell	−
CD22	B cell	−
CD23	B cell	−
CD30	Reed-Stemberg cell	−
CD34	Hemopoietic precursor	−
CD41	Megacaryocyte	−
CD43	T cell	−
CD44	Molecular adhesion	+
CD45RO	T cell	+
CD57	NK cell	−
CD61	Megacaryocyte	−
CD68	Macrophage	−
CD79a	B cell	−
β F1 T cell	TCR αβ heterodimer	−
TCR δ 1	TCR γδ heterodimer	+
EBV probe (EBER)		−

*NHL, non-Hodgkin's lymphoma; NK, natural killer; TCR, T-cell receptor; EBV, Epstein-Barr virus.

histological examination. Samples of bone marrow and spleen were embedded in metacrilate for better morphological definition. Immunohistochemical studies of paraffin and frozen section of spleen, liver, and bone marrow were performed with streptavidin-biotin technique; a panel of monoclonal antibodies was used to characterize the tumor cells (Table I). In situ hybridization for EBV encoded RNA was performed on paraffin embedded sections of the spleen, liver, and bone marrow with EBER 1 and EBER 2 oligonucleotide peptide nucleic acid probes.

The spleen weighed 2,700 g without evidence of lymph nodes at hilum and had a homogeneous cut surface, free of nodules. Histologically, it showed an expanded red pulp with atrophy of residual white pulp; the red pulp was characterized by dilated sinusoids with prominent endothelial cells and by presence of megacaryocytes, many erythroid and myeloid cells in different states of maturation, many macrophages, some of which with erythrophagocytosis (better seen by electron mycroscope), a great quantity of cellular debris and iron deposits inside and outside the macrophages, and increase of reticulin fiber focal fybrosis. Many infarcts were also present. In addition, in the red pulp there were clusters and aggregates of medium-size cell predominantly in the sinusoid but also outside in the cords (Fig. 2). These cells had round or slightly irregular nuclei, fairly ma-

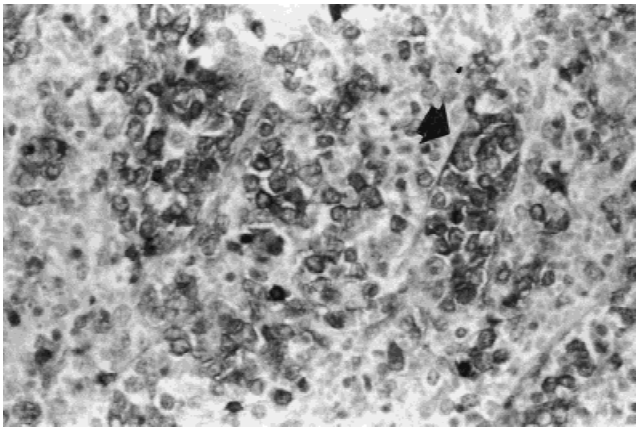

Fig. 2. Spleen: CD3+ lymphoid cells inside the vessel (arrow); the same cells are present in the cords (magnification, ×400).

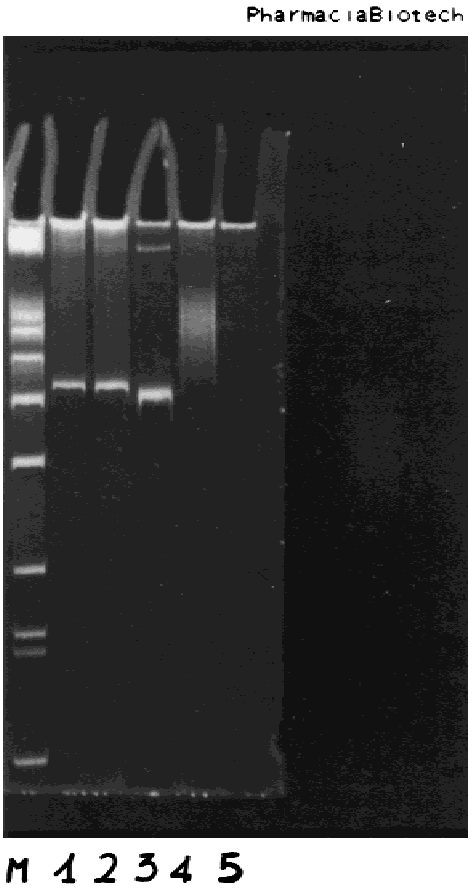

Fig. 3. Heteroduplex analysis of TCR γ gene amplification products on denaturing gradient gel electrophoresis (legend: M, molecular weight marker; 1–2, patient; 3, positive control; 4, peripheral blood mononuclear cells; and 5, H2O).

ture chromatin with inconspicuous nucleoli and different amounts of basophilic cytoplasm. The liver biopsy presented normal parenchyma with dilated sinusoids with the same atypical cells observed in the spleen. The bone marrow biopsy showed absence of adipose tissue, hypercellularity with hyperplasia of three series, increase of reticulin fibers, many macrophages, iron deposits and cellular debris, only scattered atypical cells, the same noted in the spleen, with T phenotype: CD2+, CD3+, CD7+, CD5−, CD4−, CD8−, CD44*+ (H-CAM). They did not stain with B lymphoid, erythroid, myeloid, and megacaryocytic markers. At last, to remove all doubt of lymphoma, we studied the clonal rearrangement of TCR γ locus V-J junctional region with the heteroduplex analysis of the TCR γ gene amplification products on denaturing gradient gel electrophoresis [5] modified by Bottaro et al. [6] on paraffin sections (Fig. 3).

After splenectomy pancytopenia and jaundice worsened, and retinal hemorrhage appeared. Steroid therapy (prednisone, 1 mg/Kg/day) was begun and a new bone marrow aspirate was performed; a moderate increase of lymphocytes and histiocytic cells with hemophagocytosis was noted. Chemotherapy (daunomycin, aracytin, and VP16) was started. During aplasia, the patient had septic shock and pneumonia. At restoration, hydroxyurea, 1 g per day, was administered but he deteriorated rapidly and four months after the hospital admission he died because of respiratory failure. Postmortem examination was not performed because of opposition of parents. The rearrangement of TCR γ gene was the most important result to indicate a clonal T-cell proliferation.

DISCUSSION

Postthymic T-cell malignancies are heterogeneous in their clinical and laboratory features. Among the γδ postthymic T-cell lymphomas, two distinct entities (cutaneous and hepatosplenic, respectively) are reported in the literature [1]. The former shows predominant multiple involvement of the skin and subcutaneous tissue; it occurs mostly in older patients and the phenotype is CD3−, CD4−, CD8−. Because of the small number of reports, the course of the disease was unknown. The latter shows a clinical picture characterized by hepatosplenomegaly, no or little adenopathy, and sometimes systemic symptoms (fever, cytopenias likely due to hypersplenism). The bone marrow histologic feature often reveals a little infiltration, especially sinusoidal and easily underestimated. Microanatomically, these cells appear to home to the classic T-cell zones of lymphoid tissues, particularly to splenic sinuses and cords [7]. Phenotype of this lymphoma is CD3+, CD2+, CD7+, CD4−, CD8−, CD5− [8].

The clinical picture of our case was mainly due to the hemophagocytic lymphohistiocytosis (HLH). This is a very rare disease, most frequent in children, characterized by sudden onset with persistent fever (>7 days), splenomegaly, progressive cytopenia, jaundice, coagu-

lopathy, and hemophagocytes in the reticuloendothelial system [9]. Viral infections frequently are responsible for this syndrome but in our patient, no positivity was found for hepatic virus, CMV, herpes, or HIV. EBV is probably involved in one third of HLH cases and has been correlated recently with malignancies in this syndrome because of monoclonal proliferation of EBV-containing T- or natural killer (NK) cells [10]. The recent recognition of the association of EBV with postthymic T-cell lymphomas, particularly the nasal T/NK cell lymphoma, but also AILD-like T-cell lymphoma and large T-cell lymphoma, has documented that a high percentage of these diseases are complicated by hemophagocytic syndrome which may occur simultaneously with the onset of lymphoma [11]. The production of cytokines, namely tumor necrosis factor, by EBV-infected T lymphocytes are presumed to cause the histiocytic activation and the subsequent hemophagocytic process in T lymphomas. In our patient, EBV was not found as in other reports of hepatosplenic γδ T-cell lymphoma [12,13].

Lymphoma is the main cause of HLH in adults, and a recent report of Linn et al. [14], about ten cases of this disease shows that in eight cases with fulminant clinical course, four had high-grade peripheral T-cell lymphoma, and three had suspicious lymphomatous infiltrate in bone marrow. The two cases with a less aggressive course were found to have lymphoma of the diffuse large cell and Ki-1 anaplastic type, respectively.

Only five cases of hepatosplenic γδ T-cell lymphoma with cytogenetic study are reported [1,12–14]: one normal, two with isochromosome 7q, and two with t(7; 9) (p15; p13) translocation with implication of TCR γ chain locus on chromosome 7p15 and with loss of several chromosomes, respectively. In our patient, there was involvement of chromosomes 3 and 6. Recently, Schlegelberger et al. [15] reported cytogenetic findings in 104 peripheral T-cell lymphomas: aberrant clones were demonstrated in 69% of cases, and trisomy 3 was significantly more frequent in AILDL-T (35%), T-zone lymphomas (40%), and lymphoepithelioid lymphomas (50%). Unfortunately, no cases of hepatosplenic γδ T-cell lymphoma were reported in this study.

The hepatosplenic γδ T-cell lymphoma is not an easy diagnosis in general and in particular when the histologic pattern is represented, as in our case, by moderate infiltration of atypical lymphoid cells in spleen and liver. In fact, few atypical lymphoid cells with hyperplasia of hemopoietic cells and scattered erythrophagocytosis, seen in the aspirate and bone marrow biopsy, represented a serious difficulty to confirm a suspected diagnosis of lymphoma. Even after splenectomy the histological examination put us in difficulty due to the unusual presentation; the conspicuous hyperplasia of hemopoietic series masked the small aggregates of lymphoid cells in the cords of red pulp of the spleen.

In accordance with previous reports, this type of lymphoma presented: 1. no lymphoadenopathy; 2. contemporary atypical lymphoid cells present in spleen, liver, and bone marrow with the same phenotype (CD2+, CD3+, CD7+, CD5−, CD8−, CD4−); 3. EBV negative T cells (exclusion of other types of EBV positive T-NHL); 4. macrophage but not lymphoid erythrophagocytosis; and 5. monoclonality of γ TCR (differential diagnosis with other type of pathology represented by protozoarian infection with iperplasia of the same lymphoid cells, normally located in the red pulp of the spleen, but CD5+).

In conclusion, in our patient, it was not possible to diagnose the hepatosplenic lymphoma early. A neoplastic disease, namely lymphoma, was suspected, but bone marrow biopsies, CT scan, and laparotomy with liver biopsy and splenectomy were not immediately diagnostic for a lymphoproliferative disease. Only polymerase chain reaction contributed to the correct diagnosis of this lymphoma.

Moreover, a review of the few cases reported in adults underlines that in very aggressive HLH, the presence of hidden T-cell lymphoma is possible and probable [14] and should be searched for with most accuracy.

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